

Modulation of RBC cholesterol

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Updated date: Feb 1, 2021

An abbreviated version of this protocol was published in eLIFE in Feb 2017

Variability of cholesterol accessibility in human red blood cells measured using a bacterial cholesterol-binding toxin

DOI: 10.7554/eLife.23355

Detailed protocol

Below is a protocol for modulation of RBC cholesterol as represented in the publication.

HPCD= (2-Hydroxypropyl)- β -cyclodextrin powder

PBSx buffer= PBS supplemented with 2% NCLPPS and 1mM EDTA

RBC Cholesterol Assay Protocol: Manipulate amount of cholesterol in RBCs

*Work on ice

Preparing human red blood cells:

1. Start with a 10mL human blood sample, freshly acquired (approved IRB protocol) from venipuncture (stored for up to 2 days at 4 degrees Celsius if needed). Centrifuge the sample @4000rpm for 8 min. Aspirate and discard supernatant. Resuspend the cell pellet in 1mL PBS buffer. Into 15mL centrifuge tube, pipette 300ul blood into 4.7mL PBSx buffer.
2. Centrifuge @4000rpm for 8 min.
3. Aspirate supernatant
4. Re-suspend ea pellet in 5mL PBSx.
5. Centrifuge @4000rpm for 8 min.
6. Repeat wash 1x (steps 4-5).
7. Re-suspend pellets in 5mL PBSx.

Pre-treatment with cyclodextrin:

Sample 21605		Treatment	Diluted blood (ul)	5% HPCD (ul)	2.5mM Chol/CD (ul)	PBSx buffer (ul)
1	a	0.5% HPCD	400	100	0	1500
1	b	0.5% HPCD	400	100	0	1500
2	a	0.25% HPCD	400	50	0	1550
2	b	0.25% HPCD	400	50	0	1550
3	a	0	400	0	0	1600
3	b	0	400	0	0	1600
4	a	25uM Chol/CD	400	0	20	1580
4	b	25uM Chol/CD	400	0	20	1580
5	a	50uM Chol/CD	400	0	40	1560
5	b	50uM Chol/CD	400	0	40	1560

1. Incubate indicated samples for 30 minutes on rotator at room temperature.
2. To end reaction, centrifuge samples at 4000rpm for 8 min. Aspirate supernatant. Re-suspend with 2mL PBSx. Repeat this wash 2x to remove cyclodextrin from RBCs.

How to cite: (Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Chakrabarti, R. (2021). Modulation of RBC cholesterol. Bio-protocol Preprint. bio-protocol.org/prep803.

2. Chakrabarti, R. S., Ingham, S. A., Kozlitina, J., Gay, A., Cohen, J. C., Radhakrishnan, A. and Hobbs, H. H.(2017). Variability of cholesterol accessibility in human red blood cells measured using a bacterial cholesterol-binding toxin. eLIFE. DOI: [10.7554/eLife.23355](https://doi.org/10.7554/eLife.23355)

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